Assessment of seawater pollution by heavy metals in the neighbourhood of Algiers: use of the sea urchin, Paracentrotus lividus, as a bioindicator

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The use of the sea urchin *Paracentrotus lividus* (L.) as bioindicator of the sea water quality of the Algerian coastal environment

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Abstract

This study assessed marine contamination in heavy metals in the vicinity of the Algiers metropolis by combining chemical and toxicological data using the sea urchin *Paracentrotus lividus*. Metals were analyzed in the sediment and in the sea urchin gonads. The sediment toxicity was assessed by bioassays using sea urchin larval development. This study discriminated a site near the Algiers town as highly polluted in Pb. The contamination levels appeared to be toxic for the *P. lividus* larval development. The occurrence of other metals (Fe, Cd, Cu) was poor compared with that reported in the Mediterranean Sea, with exception of Zn that showed high values in female gonads.

Keywords : Algeria, bioassays, gonads, heavy metals, sediment, *Paracentrotus lividus*.

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Introduction

The Atlantic-Mediterranean sea urchin *Paracentrotus lividus* (Lamarck) (Echinodermata : Echinoidea) is distributed from Ireland to southern Morocco, including throughout the Mediterranean Sea. This opportunistic species has an important ecological role in different ecosystems especially in Mediterranean Sea where its grazing activity can locally control the dynamics of seaweeds (see review Lozano et al., 1995) and seagrasses (see review Tomas et al., 2004). However human activities in coastal zones can alter *P. lividus* morphology, biology and demography (Harmelin et al., 1981; Delmas et Régis, 1984, Pancucci et al., 1992) and over the long-term can modify its ecological role. The sedentary habit of *P. lividus* and its sensitivity to pollutants has led to use this species as biological-biochemical indicator of local pollution (Warnau et al. 1998, Coteur et al. 2001; Bayed et al. 2005). The sensitivity of embryos and larvae of *P. lividus* prompted its use in embryotoxicity tests (Hagström and Lönning, 1973; Pagano, 1986; Warnau et al. 1996). The embryonic and larval development of sea urchins is one of the toxicity assays used in monitoring and risk assessment programmes (see review Beiras et al., 2003).

On the Algerian coasts, *P. lividus* is a dominant species of the shallow water ecosystems where it can reach 25 individuals.m^{-2} (Semroud, 1993). It colonizes different biotop like *Posidonia oceanica* and *Cymodocea nodosa* meadows, rocky substrate with photophile algae and overgrazed rocky substrate (Semroud, 1993 ; Guettaf, 2000). It lives from the low-water limit to about 10 m depth (Dieuzeide, 1933 ; Semroud, 1993).

Around the Bay of Algiers, the populations are exposed to anthropogenic disturbance. More than 35% of the national littoral population live in the Algiers metropolitan area (between Chenoua and Cap Djinet) or more than 4.3 million persons (950 habitants. km^{-2}) in a 115 km long coastal zone, This area is highly industrialized, concentrating about 25 % of the Algerian factories with about 1000 factories in 2001 (Larid, 2003; PAC, 2005) including metallurgical, chemical, pharmaceutical, building material and hydrocarbon industries and in a lower proportion mechanical, electric and electronic engineering industries and food and paper factories. A multitude of small polluting activities, official or not, spreads also along the littoral. Two rivers flow in the bay of Algiers, El Hamiz, and principally El Harrach (970 km^{-2}; 6 m^3 h^{-1}) which drain the domestic and industrial waste waters of the Algiers city. Among the wastewaters which flow in the bay (225 millions m^3 y^{-1}) only 8% are treated (PAC, 2005). The marine area under the influence of the Algiers metropolis catches the highest pollution flow of the Algerian coasts (PAC, 2003) with 100 thousand t y^{-1} of organic chemical compounds, 175 thousands t y^{-1} of suspended matter, 1 500 t y^{-1} of nitrogen and 4 thousands t y^{-1} of phosphorus (Larid, 2003). In 2004, 46 beaches of the Bay were prohibited from swimming. The sediments of Algiers Bay are highly polluted in heavy metals, hydrocarbons and organic matter. The main metal detected in the coastal water are Hg, Pb, Cu and Zn (PAC, 2003). The biological effects of the pollution on marine ecosystem are important and the recent program on the management of the coastal environment (PAC) pointed out a biodiversity decrease of 14% in the species of major ecological interest (PAC, 2005).

The purpose of the present study was to assess the marine pollution in this area by combining toxicological and chemical data using the sea urchin *P. lividus*. Embryo-larval sediment toxicity bioassays and metal contaminant analysis were used. The metal were analyzed in the sediment. The toxicity of their bioavailable fraction was detected by abnormalities in the *P. lividus* development as this species is a valuable indicator of metal contamination and can accumulate metals as a function of the contamination level of the environment (Warnau, 1998). The results of bioassays were compared to the analysis of metal contamination in the sediments and in the gonads of adult sea urchin inhabiting the sediments previously tested. Three sites were chosen in the vicinity of the Bay of Algiers according to potential sources of pollution. The information obtained will allow the ordination of the sampling stations in term of environmental quality.

Material and Methods

Sampling sites

Two stations, Algiers Beach and Tamentfoust, were located in the Bay of Algiers and another Sidi Fredj, in the North of the Bay of Bou-Ismail (east of the Bay of Algiers) (Fig. 1). The site of Algiers Beach should be the most polluted as it is the nearest of the Algiers town. This site is characterized by a habitat mixing rocks with photophile algae and degraded meadows. The site of Tamentfoust, situated in a half closed creek, is characterised by overgrazed rocks with a sparse *Posidonia oceanica* beds. The Bay of Bou-Ismail where the site of Sidi Fredj is located has pollution levels much lower than the levels of the Bay of Algiers (PAC, 2005). More distant from the very industrialized area, it encloses a marine medical centre and shows a better health meadow. But Guehioueche and Zelmat (in PAC, 2005) indicated a progressive meadow regression which could be due to the mechanical and organic disturbance caused by the medical centre.

Collection and preparation of samples

Forty sea urchins of 45-65 mm of diameter were sampled by scuba diving in March 2002 from each sites between 1 and 5 m depth. This period correspond to the maturity period of the gonads (Semroud and Kada, 1987 ; Guettaf, 2000) before the main spawning which can partly eliminate metal load in the gonad (Warnau et al., 1998).

Just after collection, the sea urchins were measured (ambital diameter) in the laboratory and dissected. The sex was determined and the gonads were dried at 60°C for 48h and stored separately in hermetically-sealed polyethylene containers. All the manipulations were performed with stainless steel instruments.

Concomitantly to the sea urchin sampling, the upper 2 cm layer of the sediments inhabited by sea urchins was collected by coring (5 cm diameter). The samples of sediments were placed into sealed polyethylene bags, carried to the laboratory on ice and immediately dried at 60°C for 48h until constant weight then stored in hermetically-sealed polyethylene containers at room temperature.

Metal analysis

The concentration of zinc (Zn), lead (Pb), cadmium (Cd), copper (Cu) and iron (Fe) were measuring in the gonads of the urchins and in the sediment according to the method described by Coteur et al. (2003). As the sediment was very heterogeneous with big gravels, only the fraction with grain sizes < 1mm was retained and considered in this study as the ‘total fraction’. Metal concentrations were also measured on the fraction with grain sizes < 63 µm. This fraction allows to estimate the organic matter content of the sediment. Pb, Cd and Cu concentrations were determined by graphite-furnace electrothermic atomic absorption spectrometry using a Varian GTA100 SpectrAA640Z spectrometer. Concentration of Zn and Fe were measured by flame atomic absorption spectrometry using a GBC 906AA spectrometer. Accuracy of the method was tested using certified reference material (*Mytilus edulis* tissues, BCR nr 278R from the Community Bureau of Reference, Commission of the European Union, Brussels, Belgium). Detection limits for Zn, Pb, Cd, Cu and Fe were 0.002, 0.014, 0.001, 0.002 and 0.004 µg of metal per mL of digested sample respectively.

The gonads of 10 males and 10 females per site were analysed. For the sediment, 3 to 6 replicates of total fraction and 0 to 3 replicates of the fraction < 63 µm were used.

Embryo-larval bioassays

The toxicity of the sediments from the previous three sites was assessed by embryo-larval bioassays in May 2003. Natural sea water used for these tests was collected in front of the marine station of Luc-sur-Mer (Normandy, France), the reference site of the Laboratory of Marine Biology of the U.L.B. (Free University of Brussels, Belgium) where the metal analysis where performed.

Sea-water was previously allowed to decant for 48 h, filtered by a 22 µm membrane and maintained at 20 ± 1°C (FSW, filtered seawater). Adult *Paracentrotus lividus* genitors were collected intertidally from a reference population inhabiting the rocky basins of Morgat (Bay of Douarnenez, Brittany, France). Individuals were transferred in the cultivation system of the marine laboratory of the U.M.H. (University of Mons-Hainaut, Belgium). For the embryotoxicity test, reference sediment was sampled at Wimereux (Nord-Pas-de-Calais, France).

The embryo-larval bioassays were performed at the laboratory of Mons-Hainaut. Those bioassays were based on the method described by Coteur *et al.* (2003). Spawning was induced by injection of 20 µl per gram of KCL 0.5N through the peristomial membrane. Gametes from 3 females and 3 males were collected in FSW and their quality (*i.e.*, general eggs' shape and sperm's motility) was checked under Olympus TO41 inverted light microscope. Eggs from each female were fertilised by the pooled sperm from the 3 males. Embryos at early gastrula stage (4-5 h after fertilization at 14°C ± 1°C) from the 3 females were mixed before to be used for the bioassays (this pooling leads to one bioassay per site using the same mixed pool of zygotes). Experiments with embryos were performed in six-well plates (Falcon, ref. 35-3046). One plate was dedicated to each sampled sediments (Alger Plage, Tamenfoust, Sidi-Fredj and Wimereux). Each well of those plates were filled with 0.1 g of dried sediment and 10 ml of FWS was added before the bioassay. One plate filled with FWS was used as control. At the beginning of the bioassay, batches of 250-300 embryos were transferred in each well. After 72 h at 14 ± 1°C, larvae were fixed by adding 1 ml of formalin (commercial solution, 35%) in each well and the plates were maintained in an oven at 70°C during 2 hours. Plates with fixed larvae were stored at 4°C. The frequency of developmental stages was scored in each well on a random sample of 100 larvae. Developmental stages were scored using an inverted light microscope according to the morphological criteria adapted from Warnau and Pagano (1994). Our larvae were classified in 4 categories: normal plutei ("Normal", N), retarded plutei presenting a delayed development ("Retarded", R), abnormal plutei with skeletal malformations and/or gut abnormalities ("Pathologic 1", P1) and embryos whose development ended at the blastula stage or the gastrula stage ("Pathologic 2", P2). On our figures, the rates of "Viable" larvae were obtained by summing the rates of "Normal" and "Retarded" larvae from each batch.

Statistical analyses

After arcsin transformation, the developmental rates of normal and viable larvae were compared using a one-way ANOVA followed by a Bonferroni's test (Zar 1996).

Dunnet’s tests were used to compare the different rates measured to their corresponding controls (Zar 1996). Significant differences were determined at the 95% level.

Contamination levels in gonads were compared by 2-way analysis of variance (ANOVA) and subsequent Tukey HSD multiple mean comparison test (effects: sex and sampling site).

The relationship between contamination in gonad and sediment and percentage of viable larvae was studied by factor analysis using the principal-component method with an extraction matrix based on Pearson correlation coefficients and the ‘’varimax’’ method of factor rotation.

Results

Contamination level in the sediment and in the sea urchin gonads

The fraction of sediment < 63 µm was more or less abundant according to the site, 0.03, 0.26, 1.56 % of the total fraction for Sidi Fredj, Tamenfoust and Algiers Beach respectively. The sediment of Algiers Beach had an obviously higher mud content.

Tables 1 and 2 present the heavy-metal concentrations in the sediment. Only the levels in the total sediment have been statistically compared because of the low level of mud in the site of Tamentfoust and Sidi Fredj leading to a too low number of replicates in these both sites. Fe, Pb and Zn concentrations in the total fraction of the sediment differed according to the sampled sites. The site of Algiers Beach was the most contaminated in Pb but the less contaminated in Fe and Zn. Fe concentrations were significantly higher in the sediment from Sidi Fredj.

Tables 3 and 4 present the heavy-metal accumulation in the sea urchin gonads. Metal concentrations in the gonads were compared according to sex and sampling site by 2-

way ANOVA. Cd and Zn concentrations were significantly higher in female than in male gonads while Cu was more concentrated in male gonads ($p<10^{-4}$). Pb and Fe concentrations did not significantly differ according to sex. Pb, Cu, and Fe concentrations in the gonads differed according to the sampled sites ($p=10^{-3}$). Pb and Cu concentrations were significantly higher in sea urchins collected respectively in Algiers Beach and Sidi Fredj (Table 3). Fe concentrations in sea urchins from Tamentfoust significantly differed from those of Algiers Beach. No interaction was significant.

Embryo-larval development

A significant reduction of the percentages of both normal and viable larvae was only observed when embryos were exposed to sediment samples from Alger Beach (Fig. 2 A, B). The low rate of normal larvae induced in response to the sediment from Alger Beach is related to an increase of the rates of abnormal plutei (P1) and retarded plutei (R) (see Table 5). The high rate of retarded plutei induced in response to the sediment from Alger Beach (Table 5) explains why the induced rate of viable plutei remains relatively high (Fig 2B.).

Integrated data analysis

The metal concentrations in sediment, in male gonads, in female gonads and percentage of viable larvae were used in the factor analysis. The variables were distributed along axes representing factors according to the correlation coefficient of the variable with the factors (Fig. 3). The first two factors accounted for 99.9 % of the total variance of the data. On the x-axis, the concentrations in Fe, Cu and Zn in the sediment were opposed to the concentrations in Cd and Pb in the sediment. The relationships between the metal level in the sediment and the metal level in the gonads was dependent on the metal. For

Pb the link was strong between sediment and the both gonads, for Cd and Cu it was high between sediment and the male gonads. On the x-axis the percentage of viable larvae was closed to the concentrations in Fe, Cu and Zn in the sediment (e.i located on the other end of the x-axis compared to the Pb and Cd contaminations in sediment and gonads). This suggests a close relationship between Pb and Cd levels in sediment and in gonads and a strongly negative interaction with these latter parameters and the percentage of viable larvae.

The y-axis comprised, at the negative end, the levels of Zn in the male and female gonads, and the level of Fe in the female gonads, and at the positive end, the levels of Cd and Cu in the female gonads. The percentage of viable larvae was located on the negative part of this axis suggesting that this parameter was negatively intercorrelated with Cd and Cu levels in the female gonads.

Discussion

The main goal of the present study was to assess the marine pollution in the vicinity of the Bay of Algiers by combining chemical and toxicological data using the sea urchin *P. lividus* as bioindicator.

No site was distinguishable from others by a generalized higher concentration of metals in the total sediments or in the sea urchin gonads. The sediments of Algiers Beach was separated from the others by the highest concentration in Pb but the lowest concentrations in Zn and Fe, the site of Sidi Fredj by the highest concentration in Fe. Algiers Beach is the most muddy site but its present a significant increase in metal contamination only for a metal, Pb, while usually the load of metal in the sediments increase along with the percentage of the fine fraction. This fact can be due to the low sediment contamination in the other metals (see below). In the gonads, Pb also isolated the population of Algiers Beach from the others. In this site, Fe was low but not different from Sidi Fredj. This latter site presented the highest concentrations in Cu. Cd did not discriminate any site.

In the sediment, Cd and Cu values can be considered in the background concentrations of the Mediterranean range. Cd values were ranged between to 0.12 to 0.76 µg g^{-1} dry wt in the total fraction of the sediment while the background concentrations of the Mediterranean were estimated between 0.05 and 1 µg g^{-1} dry wt (EEA, 1999). We note however a higher concentration in the fraction with grain sizes <63 µm of the site of Algiers Beach (1.22 µg g^{-1} dry wt). The Cu values ranged from 4.1 to 6.4 µg g^{-1} dry wt in the total fraction of the Algerian sediment and to 20.9 µg g^{-1} in the fraction with grain sizes <63 µm (Algiers Beach) and the background concentrations of the Mediterranean ranged between 5 to 30 µg g^{-1} (EEA, 1999). The Zn (between 0.02 and 0.08 µg g^{-1} dry wt) and Fe concentrations (between 7.1 and 41.5 µg g^{-1}) were very low compared to values usually observed in the Mediterranean Sea ranged from 35 to 150 µg g^{-1} for Zn (Saad et al. 1981, Hoogstraten and Nolting, 1991; Storelli et al. 2001) and > 10^3 µg g^{-1} for Fe (Saad et al. 1981, Storelli et al. 2001, Menchi et al., 2002). Only Pb values in the total fraction of the sediment of Algiers Beach (39.6 µg g^{-1}) and in the fraction with grain sizes <63 µm of Sidi Fredj (40.6 µg g^{-1}) exceeded the background concentrations of the Mediterranean range detected between 5.2 to 23.2 µg g^{-1}(EEA, 1999) or the background (4-17 µg g^{-1}) given by the reference tables of NOAA (Buchman,1999).

In the gonads, comparisons with *P. lividus* data available from the literature (Table 6) and especially the study of Warnau et al (1998), confirms the conclusions on the level of metal contamination deduced from the sediment analysis except for Zn. The gonad concentrations in Cd, Cu and Fe from the three sampling locations averaged background concentrations in *P. lividus* gonads. Only the gonad contamination in Zn from the 3 sites and in Pb from Algiers Beach were obviously higher that the Zn and Pb background concentrations for this species. The Zn concentrations, not significantly different among the sites, were high, comparable to the concentrations estimated in female gonads of *Sphaerechinus granularis* from the bay of Bay of Brest (between 190 and 700 µg g^{-1}) (Guillou et al. 2000). This bay is known to be contaminated by this metal which covers the whole roof of the town (Troadec,1995). But in the present study the Zn gonad levels appeared disproportionate with the Zn levels in sediment indicating that sediment possibly is not the most contaminated abiotic compartment. But as Zn was dominant in the gonads of the females, the results about this essential element for animal metabolism must be cautiously interpreted (Hambidge et al., 1986). On the contrary, the high Pb levels in gonads reflected the high Pb levels in the sediment. Among the compared sites, only Rabat (Morocco) presented higher concentrations due to the untreated pottery activity of this town (Bayed et al., 2005).

However when metal discriminates the site in term of contamination, the response given by the sediment can differ from that given by the gonad. The geographical Pb gradient observed in the sediment matches closely the gradient reported by the gonad accumulation, pointing out the site of Algiers Beach as the most contaminated in this metal. But Fe and Zn bioaccumulation did not discriminate the sites in the same way as Fe and Zn sediment concentrations. Three explanations can explain the differential metal pattern: i) the bioavailability of the metals is different ii) essential elements as Fe and Zn have different accumulation pattern that the other metals iii) Fe, Zn and Cu concentrations were too low in the sediment to be suitably expressed in the biological compartment. Although the two first hypothesis are plausible, the third one is clear (see above).

The high contamination level in Pb in the site of Algiers Beach expressed by the sediment and gonad analysis was confirmed by a recent study in the water and stream sediment of Oued El Harrach which flows in the Algiers Bay. Levels of 21 to 41 µg g^{-1} dry wt, close to the levels detected in the total fraction of the sediment of Algiers Beach (39.6 µg g^{-1}) were detected in the sediments in the mouth of the Oued. This pollution was probably caused by the discharge of an Algiers untreated industrial wastewater (Yoshida et al., 2005).

The results of the bioassays pointed out Algiers Beach as the only site showing toxicity on sea-urchins embryos with 24.3 % of abnormal larvae (vs 7.9 ± 2.8 % for the 3 other sites) and 47,8 % of retarded larvae (vs 22.56 ± 4.4 % for the 3 other sites). No difference was detected between the two other Algerian sites and the reference site and the control. According to the Kobayashi criteria (1991), the level of normal plutei of Algiers Beach (20.5%) expressed a strong inhibition of the sea urchin development in relation with a high disturbance. The results of these assays appeared negatively related with the Pb concentrations in the male and female gonads which were positively correlated with the Pb concentration in the total fraction of the sediment. The integrated data analysis confirms these conclusions. The high positive relationship between the rate of viable larvae and the Cu and Fe concentrations in the sediment and male gonad and the Zn concentration in the sediment would not express a positive effect of these metals on the larval development but more precisely a lack of negative effect du to the low levels of these metals. Nevertheless the position of the viable larvae suggests a negatively correlation with Cd and Cu levels in the female gonads. Although these contamination levels were low, a possible higher influence of the female gonad on the larval viability would be considered when these gonads were contaminated by metals so toxic as Cd and Cu.

The Pb pollution was not spread out as the sediment and the sea urchins of the site of Tamenfoust, a little farther from the Oued El Harrach than the site of Algiers Beach, did not present high Pb accumulation or developmental anomalies. In a semi- closed creek, this former site could be protected from the sea water and sediment flows coming from the Oued. The site of Sidi Fredj considered as less contaminated because more distant from the very industrialized area influenced by Algiers metropolis cannot be distinguished from the site of Tamenfoust according to the criteria used in this study.

In conclusion, this study confirms the link between developmental abnormalities, metal accumulations in the gonads and metal contamination in the sediment when the metal concentration in sediment is sufficiently high. It accredits the indicator value of *P. lividus*. In the present study the analysis discriminates a site and a metal, Pb, but the method could be improved by increasing the number of toxicants analysed and by extending the metal analysis to other sea urchin storage organs as the gut which can better reflect a heavy metal accumulation (Warnau et al, 1998; Guillou et al, 2000).

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Legends of figures

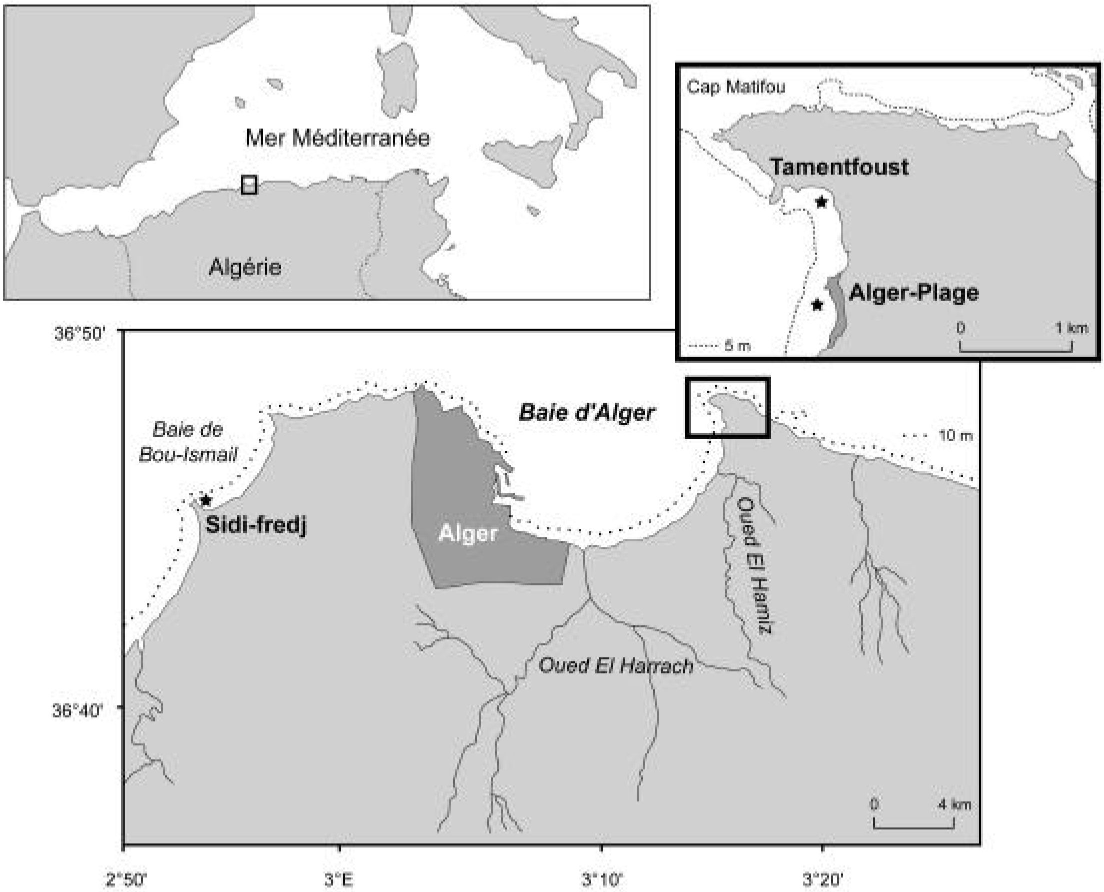

Figure 1 : Location of the three sampling sites: Algiers Beach, Tamenfoust and Sidi Fredj.

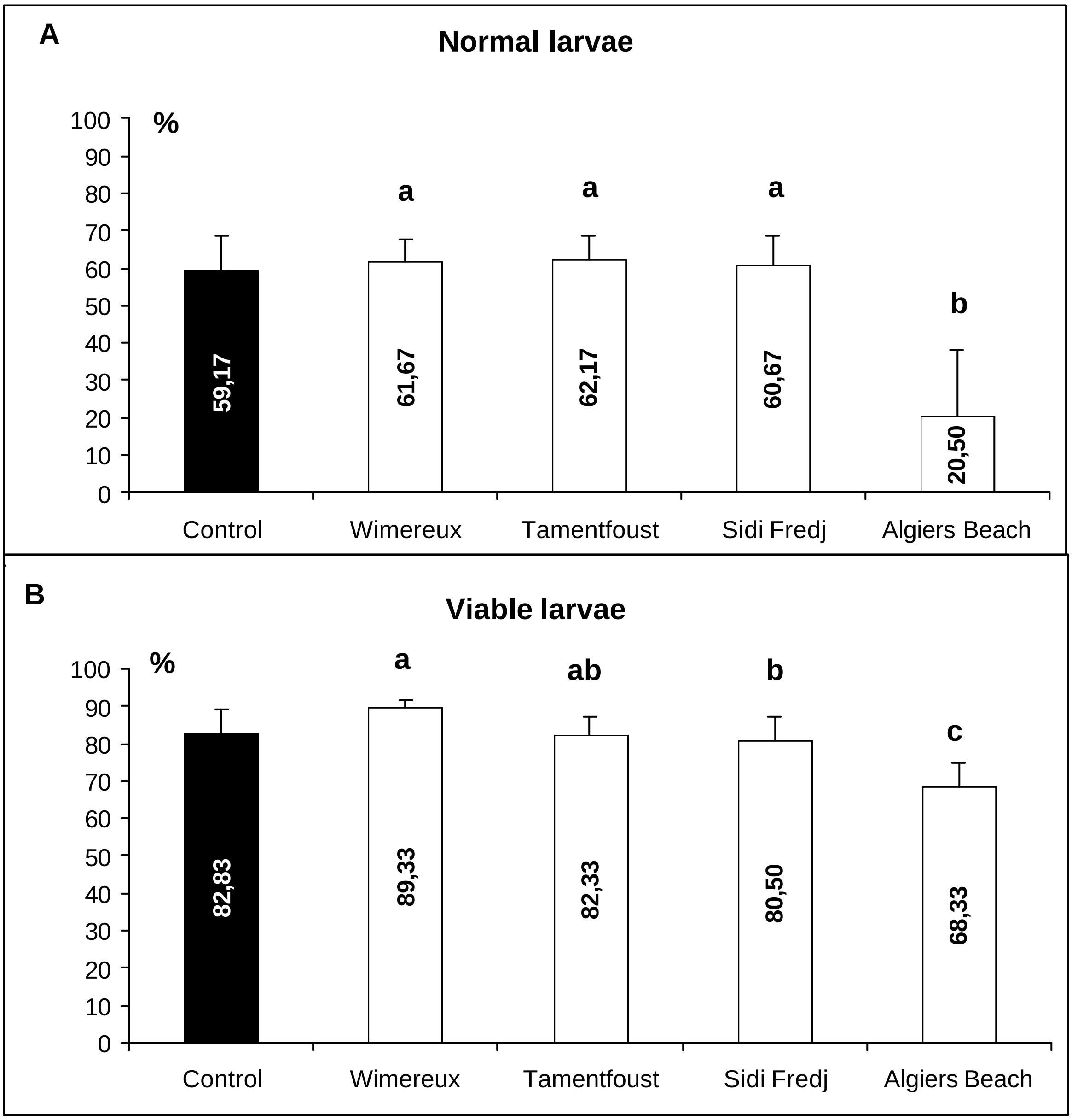

Figure 2. Percentages (mean ± S.D.) of normal (**A**) and viable (**B**) larvae of *Paracentrotus lividus* after exposition to dried sediments and control throughout embryogenesis (72 h). 6 replicates by exposure, 100 larvae scored by replicate. For each species, there were no significant differences between the series designed by the same letter (Bonferroni, p = 0.05).

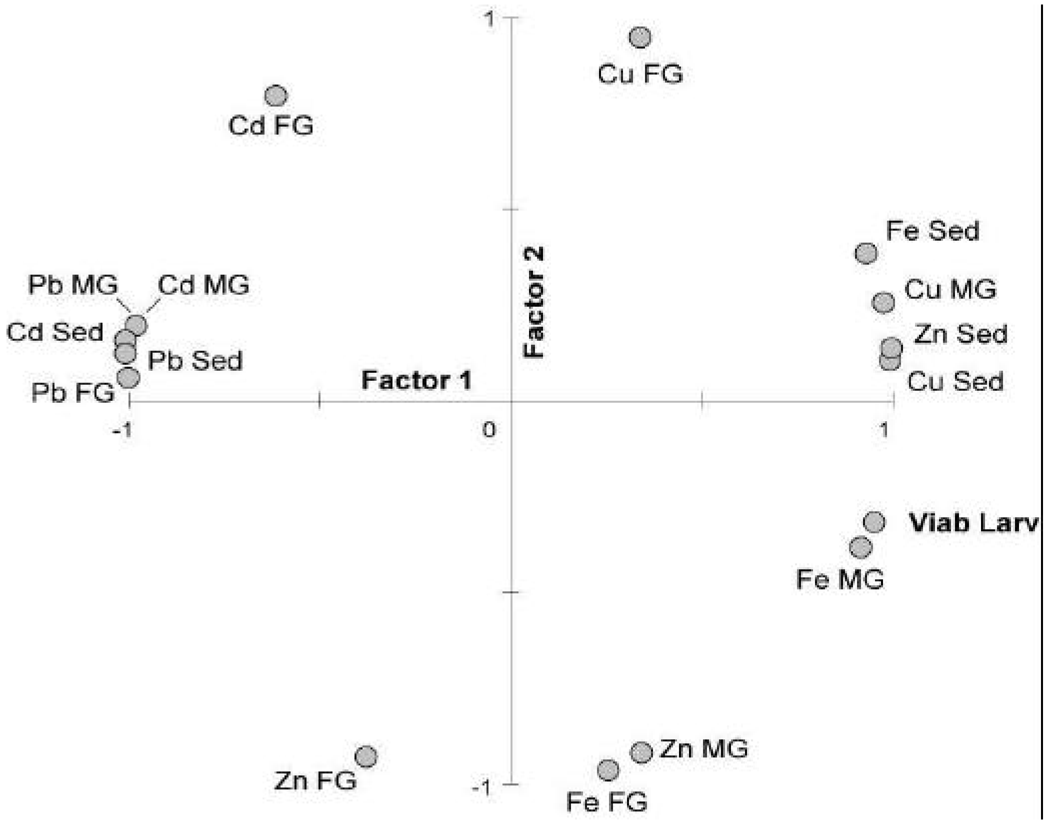

Fig. 3. Factor analysis (principal-component method) showing the relationships between the metal concentrations in the sediment (metal and suffix SED), in the male gonads (metal and suffix MG), in the female gonads (metal and suffix FG) and the percentage of viable larvae (VIABLARV). The first (x-axis) and the second (y-axis) factors accounts 70% and 29.9%. of the total variance respectively.

Legends of tables

Table 1. Metal concentration (mean ± SD ; µg g^{-1} dw) in the total fraction (n =3 to 6) and in the <63µm grain-size fraction (n= 0 to 3) of the sediment collected in the three Algerian sites (AB: Algiers Beach; TM: Tamenfoust; SF: Sidi Fredj) .

Metals in sediments		Zn	Pb	Cu	Cd	Fe
Total fraction						
AB	Means	0.023	39.63	4.08	0.76	7.11
	SD	0.005	7.93	0.62	1.05	2.18
TM	Means	0.045	14.59	5.73	0.15	17.85
	SD	0.010	1.55	1.65	0.06	3.71
SF	Means	0.050	12.37	6.43	0.12	31.32
	SD	0.006	4.07	1.32	0.01	6.49
<63 µm fraction						
AB	Means	0.081	23.76	20.89	1.22	19.70
	SD	0.008	0.75	0.75	0.04	2.08
SF	Means	0.08	40.63	10.48	0.25	41.50
	SD	-----	------	------	-----	-----

Table 2. Comparison of the metal concentrations in the total fraction of the sediments measured in the three Algerian sites (AB: Algiers Beach; TM: Tamenfoust; SF:Sidi Fredj) .

Metal in sediments Total fraction	*p* ANOVA	Level of contamination [b] + → -
Zn	0.03	SF TM AB
Pb	$> 10^{-2}$	AB TM SF
Cu	NS [a]	**TM SF AB**
Cd	NS	AB TM SF
Fe	$> 10^{-2}$	SF TM AB

[a] NS, no significant difference
[b] stations which do not differ in metal concentration are underlined (p >0.05, Tukey HSD test)

Table 3. Metal concentrations (mean ± SD ; µg g^{-1} dw; n=10) in the gonads of *Paracentrotus lividus* collected in the three Algerian sites (AB: Algiers Beach; TM: Tamenfoust; SF: Sidi Fredj) .

Metals in gonads		Zn	Pb	Cu	Cd	Fe
AB						
Females	Means	385.5*	6.14	2.84	0.14	73.8
	SD	344.1	3.46	0.97	0.08	35.5
Males	Means	32.9	7.78	3.19	0.08	19.3
	SD	13.5	8.77	0.83	0.04	19.7
TM						
Females	Means	538.2*	1.5	2.49*	0.12	113
	SD	324.3	1.72	0.47	0.08	37.6
Males	Means	76.1	0.88	3.88	0.05	112.6
	SD	172.2	0.44	0.84	0.01	66
SF						
Females	Means	366.9	0.68	3.42	0.14*	71.1
	SD	178.3	0.12	0.85	0.09	54.8
Males	Means	52.9	0.90	4.42	0.05	92.7
	SD	73.2	0.41	0.56	0.03	78.8

* indicates a significant difference between the sexes

Table 4 Comparison of metal concentrations in the gonads of *Paracentrotus lividus* measured in the three Algerian sites (2-factor ANOVA: sex and site) (AB: Algiers Beach; TM: Tamenfoust; SF:Sidi Fredj) .

Metal in gonads	Sex	*p* ANOVA Site	Interaction	n	Level of contamination [b] + –
Zn	$<10^{-4}$	0.26[a]	0.53	60	**TM SF AB**
Pb	0.69[a]	$<10^{-4}$	0.66	60	**AB TM SF**
Cu	$<10^{-4}$	$<10^{-2}$	0.11	60	**SF TM AB**
Cd	$<10^{-4}$	0.45[a]	0.80	60	**SF AB TM**
Fe	0.40[a]	$<10^{-3}$	0.06	60	**TM SF AB**

[a] no significant difference
[b] stations which do not differ in metal concentration are underlined (p >0.05, Tukey HSD test)

Table 5. Frequencies (means ± standard errors) of developmental stages in *Paracentrotus lividus* larvae exposed to sediments samples throughout embryogenesis.

	N	R	P1	P2	Du1	V	Du2
Control	59.2 ± 9.7	23.7 ± 5.9	8.7 ± 4.7	8.5 ± 3.2		82.8 ± 6.3	
					= 0.6		= 0.05
Wimereux	61.7 ± 5.8	27.7 ± 6.2	5.0 ± 2.3	5.7 ± 2.2		89.3 ± 2.4	
					= 0.6		= 0.4
Sidi Fredj	60.7 ± 8.1	19.8 ± 2.6	10.7 ± 3.1	8.8 ± 4.2		80.5 ± 6.8	
					= 0.6		= 0.5
Tamentfoust	62.2 ± 6.6	20.2 ± 3.9	8.2 ± 3.3	9.5 ± 2.6		82.3 ± 4.8	
					< 0.0005		< 0.0005
Algiers Beach	20.5 ± 17.4	47.8 ± 13.5	24.3 ± 7.9	7.3 ± 3.3		68.3 ± 6.4	

N: normal plutei; R: retarded plutei; P1: abnormal plutei; P2; Blastula; V: viable plutei; Du1: result of the Dunnett test comparing the rate of normal plutei to the control; Du2: result of the Dunnett test comparing the rate of viable plutei to the control.

Table 6. Comparison of the mean concentrations (µg g^{-1} dw) of metals in the gonads of *Paracentrotus lividus*..

Authors (sampling date)	Sites	Zn	Pb	Cu	Cd	Fe
Present study March 2002	**Algiers Beach**					
	Females	385.5	**6.14**	2.84	0.14	73.8
	Males	32.9	7.78	3.19	0.08	19.3
	Tamentfoust					
	Females	**538.2**	1.5	2.49	0.12	113
	Males	76.1	0.88	3.88	0.05	112.6
	Sidi Fredj					
	Females	366.9	0.68	3.42	0.14	71.1
	Males	52.9	0.90	4.42	0.05	92.7
Bayed et al 2005 March 2000	Atlantic Morocco					
	Rabat					
	Females		**35.32**	3.34	**25.15**	
	Males		12.41	0.52	2.58	
	Bouznika					
	Females		**11.3**	**5.56**	2.21	
	Males		7.18	1.52	1.51	
	Mohammedia					
	Females		5.22	2.51	2.24	
	Males		6.14	1.18	2.32	
Warnau et al 1998	Calvi	124	2.25	0.15	3.47	51
	Ischia	140	3.02	0.41	3.41	90
	Marseille	109	3.68	0.19	3.51	39
Storelli et al 2001 April 1998	Adriatic Sea	157.1	0.86	0.24	5.19	18.37

The higher values are in bold and underlined